

Pellicori, P., Goode, K. M., Nicholls, R., Ahmed, D., Clark, A. L., and Cleland, J. G.F. (2016) Regional circulatory distribution of novel cardiac bio-markers and their relationships with haemodynamic measurements. *International Journal of Cardiology*, 210, pp. 149-155.
(doi:10.1016/j.ijcard.2016.02.081)

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Deposited on: 19 October 2016

Word count: 2144 (text), 197 (abstract)

Regional Circulatory Distribution of Novel Cardiac Bio-Markers and Their Relationships with Haemodynamic Measurements

Short title: regional circulatory biomarker distribution

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Conflict of interest: JGFC has received research funding from Thermo Fisher Scientific (formerly B.R.A.H.M.S) and RN was employed using this research funding.

Abstract

Background: Regional sampling may identify sites of production or removal of novel biomarkers in the circulation; their relationship to haemodynamic measurements may clarify their association with the pathophysiology of heart failure.

Methods: Samples were obtained from up to eight circulatory sites from 22 patients with left ventricular dysfunction undergoing elective cardiac catheterisation. The plasma concentrations (PC) of six biomarkers [mid-regional pro-atrial natriuretic peptide (MR-proANP), C-terminal pro-endothelin-1 (CT-proET-1), mid-regional pro-adreno-medullin (MR-proADM), pro-calcitonin (PCT), copeptin and galectin-3 (Gal-3)] were measured.

Results: Plasma concentrations of MR-proANP were highest in the pulmonary artery (PA) and left ventricle, suggesting myocardial production. Lower concentrations of copeptin, CT-proET-1, MR-proADM and PCT were found in the supra-renal inferior vena cava (SRIVC) sample suggesting renal extraction. Plasma concentrations of Galectin-3 varied little by sampling site. Plasma concentrations of MR-proANP ($r=0.69$, $p=0.002$), MR-proADM ($r=0.51$, $p=0.03$), CT-proET-1 ($r=0.60$, $p=0.009$) and Copeptin ($r=0.47$, $p<0.05$) measured from PA samples correlated with PA systolic pressure. There was no relation between any measured marker and cardiac index.

Conclusions: Regional sampling shows variation in the plasma concentration of various novel peptides that provides clues to sites of net production and removal. Plasma concentrations of several biomarkers were positively correlated with pulmonary artery pressure.

regional circulatory bio-marker distribution

Keywords: left ventricular dysfunction, heart failure, biomarker, pulmonary artery pressure, cardiac haemodynamics

Introduction

Cardiac biomarkers, such as natriuretic peptides, have an established role in the diagnosis and risk stratification of heart failure (HF) (1-5). Other biomarkers may reflect the diverse pathological processes, in the cardiovascular and other systems, which contribute to the development and progression of HF and are potential targets for novel treatments. Plasma concentrations of biomarkers reflect the net balance between production and clearance but cannot distinguish between these two. Little is known about sites of production or disposal of many biomarkers or their relationships with haemodynamics, which could help explain their associations with adverse outcome (6-10).

We sought to evaluate regional differences of six novel biomarkers sampled across multiple sites in the arterial and venous circulation to explore likely sites of production and disposal. We also studied their association with haemodynamic measures of left and right heart function. The biomarkers were chosen to reflect different pathways potentially involved in heart failure: mid-regional pro-atrial natriuretic peptide (MR-proANP), a stable fragment of the ANP prohormone which reflects atrial pressure or transmural stress (11-12); mid-regional pro-adrenomedullin (MR-proADM), a vasodilator peptide that may also offer cytoprotection (13-15); C-terminal pro-endothelin-1 (CT-proET-1), which mirrors the production of endothelin, a potent vasoconstrictor reflecting endothelial dysfunction (16-17); C-terminal pro-arginine vasopressin (CT-proAVP or copeptin), a measure of AVP production (18); ultrasensitive procalcitonin (hsPCT), a marker of infection (19-22); and galectin-3, a measure of tissue injury and fibrosis (23-24). All of these markers

are elevated in heart failure and, in some way, reflect the severity of disease and prognosis (6-9).

Methods

Study Population

Twenty-two patients with left ventricular dysfunction (either a reduced left ventricular ejection fraction [LVEF <50%] or an increased left atrial diameter [LAD \geq 4.0cm] on two-dimensional echocardiography) or a raised left ventricular end-diastolic pressure [LVEDP >16mmHg] invasively measured) undergoing elective left and right heart catheterisation were enrolled (seven patients had significant valvular disease, nine had ischemic heart disease (IHD), one had both severe mitral regurgitation and IHD, two had an atrial septal defect and one had complete heart block with superior vena cava obstruction). The mean age was 65 (\pm 12) years (ranging from 30 to 83), thirteen (59%) were men, seven (32%) had atrial fibrillation, one had chronic lung disease, 19 had reduced functional capacity due to breathlessness, five had a left ventricular ejection fraction <40% and 62% were treated with loop diuretics (table 1).

The investigation conformed to the principles outlined in the Declaration of Helsinki and all subjects gave their written informed consent.

Cardiac Catheterization

Cardiac catheterisation was performed in the afternoon with patients in a fasting state. Catheters were inserted via the femoral artery and vein to measure: pulmonary artery (PA), pulmonary capillary wedge (PCWP), right atrial, systemic arterial and LV end-diastolic pressures. Cardiac output was measured by the direct Fick method. Venous samples were collected from up to five circulatory sites: the low inferior vena cava (LIVC), supra -renal inferior vena cava (SRIVC), high inferior vena cava (at the level of the hepatic vein; HIVC), superior vena cava (SVC) and pulmonary artery (PA). Arterial samples were collected from the left ventricle (LV) and aortic root. In five cases, samples were also obtained from the coronary sinus. After samples were collected, all patients underwent coronary arteriography and left ventriculography.

Plasma concentrations were compared using aortic samples as reference. In addition, an estimate of net cardiac production or removal was made by comparing biomarker concentrations in the PA with calculated mixed venous blood using the formula (mixed venous concentration = $((3 \times \text{SVC}) + \text{HIVC})/4$). Further comparisons were made to explore net renal (LIVC to SRIVC), hepatic (SRIVC to HIVC) and lung (PA to LV) production or removal.

Samples were collected into ethylene-diamine-tetra-acetic acid (EDTA) vacutainers. The vacutainers were centrifuged immediately after collection at 3000 rpm for 15 minutes at 4°C. The plasma was removed from each and stored in a cryotube at -80°C prior to batch analysis. The plasma concentrations (PC) of MR-proANP, CT-proET-1, MR-proADM, hsPCT and copeptin were measured using a kryptor analyser (B.R.A.H.M.S. AG, Henningsdorf, Germany), a fully automated system based on time-resolved amplified cryptate emissions (TRACE) technology. Galectin-3 was

measured using a manual enzyme-linked immuno-sorbent assay (BG Medicine, Waltham MA, USA).

All samples were measured in duplicate and the average value of the two measurements was used.

The lower limits of detection for these assays were 2.1 pmol/L for MR-proANP, 0.05 nmol/L for MR-proADM, 3.0 pmol/L for CT-proET-1, 4.8 pmol/L for copeptin and 7 ng/L for ultrasensitive PCT. The functional assay sensitivities, defined as the concentration at which the inter-assay coefficient of variation (CoV) was 20%, were 10 pmol/L for MR-proANP, 0.25 nmol/L for MR-proADM, 10 pmol/L for CT-proET-1, 12 pmol/L for copeptin and 30 ng/L for ultrasensitive PCT.

Statistical methods

Regional differences were expressed as the mean and percentage change. The plasma concentrations of biomarkers were log transformed if they were not normally distributed. Where samples were missing, comparisons were made only where there was a sample in both regions. The differences in the mean assay values between each of the circulatory sites were compared using the paired t-test for related samples. Formal adjustments for multiple comparisons were not made as the study was exploratory to generate rather than prove a hypothesis. The correlations between plasma concentrations of different biomarkers and haemodynamic measurements were examined using Pearson correlation coefficient and scatter plots for samples

obtained in the aorta and in the pulmonary artery. Due to the exploratory nature of this study, statistical significance was set at a p-value <0.1.

Results

Patient characteristics

Demographic and echocardiographic characteristics, medications and blood results for the study population are reported in Table 1.

Differences in regional circulatory distribution and relationship with haemodynamic measurements (Figures 1 to 6 and Table 2).

MR-proANP. Plasma concentrations of MR-proANP fell between aorta and SRIVC, suggesting renal extraction, and increased again in the pulmonary artery suggesting a contribution from the coronary sinus and/or right heart (figure 1). The drop in concentration between the aorta and the SRIVC was close to 10% ($p=0.0004$) and there was a significant relationship between eGFR and this difference, again suggesting renal clearance (Figure 7). MR-proANP measured from PA samples correlated with age [$R=0.59$; $P=0.010$] and systolic PA [$R=0.69$; $P=0.002$] and mean PA [$R=0.78$; $P<0.001$] and end diastolic left ventricular filling [$R=0.55$; $P=0.02$] pressures.

MR-proADM. Plasma concentrations of MR-proADM increased between the aorta and the low IVC, consistent with production by the capillary endothelium, fell in the SRIVC, suggesting extraction in the renal circulation, and then rose again in HIVC, suggesting a contribution from the splanchnic circulation (Figure 2). Plasma concentrations measured in the PA correlated with age [$R=0.66$; $P=0.003$] and systolic PA [$R=0.51$; $P=0.03$] and mean PA [$R=0.56$; $P=0.02$] pressures.

CT-proET-1. Plasma concentrations of CT-proET-1 decreased from the LV to the aorta, where there was a statistically significant step down; the explanation for this is not clear (Figure 3). CT-proET-1 concentrations measured in the PA correlated with age [$R=0.71$; $P=0.001$] and systolic PA [$R=0.60$; $P=0.009$], mean PA [$R=0.54$; $P=0.02$] and RA [$R=0.58$; $P=0.01$] pressures and weakly with cardiac index.

Copeptin. Plasma concentrations of copeptin dropped between the aorta and SRIVC and then increased in the HIVC (Figure 4). Copeptin measured in the PA correlated with age [$R=0.47$; $P=0.051$] and systolic PA pressure [$R=0.47$; $P=0.048$].

Ultrasensitive pro-calcitonin. Plasma concentrations of pro-calcitonin fell between aorta and SRIVC and rose again in the HIVC (Figure 5). No correlations were found between pro-calcitonin measured in the PA and haemodynamic measurements.

Galectin-3. Plasma concentrations of Galectin-3 plasma were similar in the right and left circulation, although its plasma concentrations decreased between LIVC and SRIVC, suggesting renal clearance (Figure 6). Galectin-3 measured in the PA correlated with eGFR [$R=-0.54$; $P=0.02$] and weakly with mean PA pressure, but there were no other correlations between galectin-3 concentration and haemodynamic measurements.

Samples were obtained from the coronary sinus in only five patients. These suggested a net contribution for MR-proANP (8.6%), CT-proET-1 (6.5%) and hsPCT (5.8%) but no net contribution from other markers (0% - 1.0%).

Discussion

This study provides insights into the sites of production and clearance of novel cardiac biomarkers. We observed a general pattern for the majority of the biomarkers: their plasma concentrations were often lower in the SRIVC compared to the aorta, suggesting renal clearance, were higher when PA pressures were raised and were unrelated to cardiac index, systemic blood pressure or systemic vascular resistance.

MR-proANP is a fragment of the A-type natriuretic peptide pro-hormone produced by cardiomyocytes in response to pressure or fluid overload. ANP enhances natriuresis and may be considered part of the body's defence against congestion. The highest plasma concentrations were found in the aorta and pulmonary artery, consistent with cardiac production. Of the biomarkers we measured, only MR-proANP was related to LVEDP (11); lower concentrations in the SRIVC suggest renal extraction (12).

Adrenomedullin is a vasodilator peptide that was originally discovered in a pheochromocytoma. (13) It is produced by the endothelium at a rate similar to that of endothelin-1 in cultured vascular endothelial cells (14). The drop between the aorta and SRIVC suggests renal extraction. The increase at the HIVC level suggests that the adrenal glands or splanchnic circulation might contribute to its production. A similar pattern was shown for CT-proET-1, although changes were less marked. No net change across the pulmonary circulation was observed for either marker (15, 16) but their association with pulmonary artery pressures suggests that the pulmonary circulation might have a role in their turnover (17).

Copeptin is an inactive fragment of a pre-prohormone synthesised in the hypothalamus and co-secreted with vasopressin from the posterior pituitary. The highest plasma concentrations were in the superior vena cava consistent with its known site of production. Although it is unknown how copeptin is removed from the circulation, concentrations were lower in the SRIVC, suggesting renal extraction. Copeptin degradation fragments have been detected in urine. (18).

Procalcitonin (PCT) is a biomarker related to the presence and severity of infection (19) but plasma concentrations may be modestly elevated in HF even when evidence of infection is absent (20). Raised plasma PCT concentrations are associated with a worse prognosis (21). Patients with heart failure may have altered gut permeability due to bowel congestion and this may lead to absorption of bacterial endotoxins stimulating PCT release (22). In our population, PCT was not associated with the haemodynamic severity of disease. Again, we found evidence of renal extraction of

PCT. The rise in PCT in the HIVC suggests that liver and other abdominal organs might be extra-thyroidal sites of PCT production.

Galectin-3 promotes macrophage migration, fibroblast proliferation and collagen synthesis, all of which are important steps in the genesis of myocardial fibrosis (23). In animal models, high galectin-3 promotes cardiac fibrosis and ventricular dysfunction (24). We found no substantial variability in galectin-3 other than a drop between LIVC and SRIVC, again suggesting renal extraction (25).

Current evidence suggests that measurements of right rather than left heart function may be the more important determinant of prognosis in patients with HF; an elevated pulmonary pressure may play a key role in the development of RV dysfunction (26-28). Plasma concentrations of most of the biomarkers we measured correlated with PA pressure which might explain some of the association between biomarker concentrations and outcome in patients with HF (6-9).

Limitations

The present study can only be considered exploratory due to its size. Verification by others would be welcome. The half-lives of these biomarkers in the circulation are unknown but many are relatively stable fragments with relatively low clearance rates. Net changes across a circulatory bed may be difficult to observe when turnover is low. We investigated only net changes and not turnover that would have required isotopic labelling of markers. It is possible that production and extraction of a biomarker may be balanced across a circulatory bed; under conditions other than those in our study, this balance may be disturbed. We did not take a peripheral blood

sample from an antecubital vein and so we cannot be sure of the relationship between our findings and plasma concentrations in samples that would be obtained in routine practice. We did not specifically cannulate and obtain samples from the renal or splanchnic veins that might have demonstrated more pronounced changes. This population was heterogeneous with only five patients having substantial LV systolic dysfunction. Moreover, patients were recruited from a cohort of patients undergoing elective left and right heart catheterization, thus these results do not apply to patients who are acutely ill. Patients were studied under artificial conditions (supine position; absent muscle activity). There were several minutes between acquisition of the first and last sample, which might have affected our results.

Conclusions

This study shows that regional circulatory differences exist in plasma concentrations of many biomarkers, that extraction across the renal circulation is a common feature for many and that their plasma concentrations rise when PA pressures increase; presumably reflecting congestion. This may explain why elevated plasma concentrations are associated with adverse prognosis.

Disclosures: JGFC has received research funding from Thermo Fisher Scientific (formerly B.R.A.H.M.S) and RN was employed using this research funding.

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Legend to figures

Figure 1 to 6: Differences in the mean plasma concentration for each of the circulatory sites compared with that from aortic samples (reference site). Suprarenal inferior vena cava (SRIVC), low inferior vena cava (LIVC), high inferior vena cava (HIVC), superior vena cava (SVC) and coronary sinus when available (CS). To further explore the net organ contribution, comparison between LIVC and SRIVC (renal), SRIVC and HIVC (splanchnic), pulmonary artery (PA) and left ventricle (LV; lungs), Mixed venous $((3 \times \text{SVC}) + \text{HIVC})/4$ and PA (cardiac), and finally LV and Aorta were made. Where samples were missing comparisons were made only where there was a sample in both regions.

Figure 7: relationship between estimated Glomerular Filtration Rate (eGFR) and the changes in MR-proANP (%) between aorta and suprarenal inferior vena cava.

Patient Characteristics	
Demographic	
Age – years	65 (12)
Male – no. (%)	13 (59)
IHD - no. (%)	10 (45)
Diabetes - no. (%)	2 (9)
Hypertension - no. (%)	4 (18)
COPD - no. (%)	1 (4)
Atrial fibrillation- no. (%)	7 (32)
NYHA class – no. (%)	
I	3 (14)
II	18 (82)
III	1 (4)
BMI – kg/m ²	25 (7)
Heart rate – bpm	75 (15)
Blood results	
Haemoglobin – g/dl	13.6 (1.8)
Creatinine – umol/l	89 (19)
Urea – mmol/l	6.1 (4.1 – 7.5)
eGFR - ml/min/1.73m ²	77 (18)
Echocardiographic Data	
LVEF - %	55 (41-60)
LVEF <40% – no. (%)	5 (23)
LAD – mm	45 (10)
Moderate or Severe mitral regurgitation	5 (23)
Moderate or Severe tricuspid regurgitation	8 (36)
Medications – no. (%)	
Beta-blocker	15 (67)
ACE inhibitor or ARB	11 (50)
Loop Diuretics	14 (63)
Aldosterone Antagonists	7 (32)

Table 1: Patient Characteristics. The data shown is mean and standard deviation if the variable is normally distributed or median and inter-quartile range if not. List of abbreviations used: IHD - Ischaemic Heart Disease; COPD - Chronic Obstructive Pulmonary Disease; BMI - Body Mass Index; eGFR - estimated Glomerular Filtration Rate; LVEDD - Left Ventricle End-Diastolic Diameter; LVEF – Left Ventricular Ejection Fraction; LA - Left Atrial Diameter.

	Median Values in PA (IQR)	Age years	BMI kg/m ²	eGFR ml/min/ 1.73m ²	RAP- mmHg	SPAP- mmHg	MPAP mmHg	PVR dyn·s/cm ⁵	PCWP mmHg	LVEDP mmHg	CI l/min/m ²	SAP mmHg	SVR dyn·s/cm ⁵
		Median (IQR)											
		66 (57-75)	25 (21-29)	77 (67-88)	8 (4-12)	41 (29-51)	28 (19-34)	233 (32-310)	19 (10-25)	15 (9-22)	1.81 (1.65-2.25)	125 (106-162)	1623 (1433-2344)
		Pearson's Correlation, r (p-value)											
MR-proANP* pmol/l	232 (149-325)	0.590 (0.01)	-0.186 (0.46)	-0.254 (0.31)	0.244 (0.33)	0.690 (0.002)	0.780 (<0.001)	0.262 (0.34)	0.337 (0.22)	0.545 (0.02)	-0.208 (0.41)	0.045 (0.86)	0.184 (0.46)
MR-proADM nmol/l	0.76 (0.60-1.06)	0.658 (0.003)	-0.007 (0.98)	-0.376 (0.12)	0.460 (0.05)	0.513 (0.03)	0.561 (0.01)	0.239 (0.39)	0.216 (0.44)	0.314 (0.22)	-0.248 (0.32)	-0.213 (0.37)	-0.078 (0.76)
CT-ProET1 pmol/l	93 (68-102)	0.712 (0.001)	-0.334 (0.17)	-0.368 (0.13)	0.582 (0.01)	0.595 (0.009)	0.544 (0.02)	0.199 (0.47)	0.327 (0.24)	0.111 (0.67)	-0.439 (0.07)	-0.227 (0.36)	0.014 (0.96)
Copeptin* pmol/l	10.3 (7.8-16.5)	0.466 (0.05)	-0.101 (0.69)	-0.415 (0.08)	0.368 (0.13)	0.472 (0.05)	0.365 (0.14)	0.455 (0.09)	-0.120 (0.67)	0.100 (0.70)	-0.273 (0.27)	-0.271 (0.28)	-0.117 (0.64)
Procalcitonin* ng/L	29.8 (18.0-40.3)	0.363 (0.14)	-0.248 (0.32)	-0.022 (0.93)	0.405 (0.09)	0.371 (0.13)	0.158 (0.53)	0.240 (0.39)	-0.133 (0.64)	-0.211 (0.42)	-0.173 (0.49)	-0.264 (0.29)	0.074 (0.77)
Galectin-3 ng/ml	17.8 (12.3-21.4)	0.398 (0.10)	0.043 (0.86)	-0.541 (0.02)	0.127 (0.62)	0.384 (0.11)	0.439 (0.07)	-0.074 (0.79)	0.205 (0.46)	-0.038 (0.88)	0.046 (0.86)	-0.034 (0.89)	-0.187 (0.46)